# Two Loci, *Tmevp2* and *Tmevp3*, Located on the Telomeric Region of Chromosome 10, Control the Persistence of Theiler's Virus in the Central Nervous System of Mice

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ABSTRACT

Theiler's virus persistently infects the white matter of the spinal cord in susceptible strains of mice. This infection is associated with inflammation and primary demyelination and is studied as a model of multiple sclerosis. The *H-2D* gene is the major gene controlling viral persistence. However, the SJL/J strain is more susceptible than predicted by its *H-2*$^s$ haplotype. An (SJL/J × B10.S)$F_1$ × B10.S backcross was analyzed, and one quantitative trait locus (QTL) was located in the telomeric region of chromosome 10 close to the *Ifng* locus. Another one was tentatively mapped to the telomeric region of chromosome 18, close to the *Mbp* locus. We now report the study of 14 congenic lines that carry different segments of these two chromosomes. Although the presence of a QTL on chromosome 18 was not confirmed, two loci controlling viral persistence were identified on chromosome 10 and named *Tmevp2* and *Tmevp3*. Furthermore, the *Ifng* gene was excluded from the regions containing *Tmevp2* and *Tmevp3*. Analysis of the mode of inheritance of *Tmevp2* and *Tmevp3* identified an effect of sex, with males being more infected than females.

THE DA strain of Theiler's virus, a picornavirus, causes a chronic demyelinating disease in genetically susceptible mice and is a model for multiple sclerosis (Miller and Gerety 1990; Drescher *et al.* 1997; Monteyne *et al.* 1997). During the first two weeks that follow intracranial inoculation, the virus infects mainly neurons of the brain and spinal cord. Although some mice may suffer from mild flaccid paralysis of the hind legs, all of them recover from this acute encephalomyelitis. In susceptible animals, this early disease is followed by a lifelong persistent infection of glial cells in the white matter of spinal cord. Persistence is accompanied by chronic inflammation and primary demyelination, which are sometimes responsible for gait disorders and spastic paralysis (Brahic *et al.* 1981; Lipton *et al.* 1984). In contrast, in genetically resistant mouse strains, recovery from the early disease coincides with clearance of the virus. As a result, resistant mice do not show inflammation or demyelination in their spinal cord.

Susceptibility to persistent infection and to demyelination differs greatly among strains of mice and is under the control of several genes, including the H-2D region of the major histocompatibility complex (MHC; Brahic and Bureau 1998). Because viral persistence is a prerequisite for demyelination and because demyelination is a complex phenotype that is not easily quantified, we chose to study the genetics of susceptibility to persistent infection by using a semi-quantitative assay for the amount of viral RNA in the spinal cord 45 days after inoculation. At this time, the virus has been eliminated from the central nervous system (CNS) of resistant mice and is causing extensive demyelination in susceptible ones. The phenotype of all inbred strains studied can be explained by the interaction of two groups of loci (Bureau *et al.* 1992). One locus, Theiler's murine encephalomyelitis virus persistence 1 (*Tmevp1*), is in the H-2D region of the MHC. The *q* haplotype is associated with high susceptibility, the *b* haplotype with resistance, and the *d*, *k*, and *s* haplotypes with intermediate susceptibility. The resistant *b* haplotype is dominant. *Tmevp1* has been shown recently to be the *H-2D* gene itself (Azoulay *et al.* 1994; Lipton *et al.* 1995; Rodriguez and David 1995). The SJL/J strain is an exception to this classification because it is more susceptible than predicted by its *H-2*$^s$ haplotype. This is illustrated by comparing the SJL/J and B10.S strains that bear the *H-2*$^s$ haplotype but are, respectively, highly susceptible and of intermediate susceptibility. Their $F_1$ cross is susceptible, which shows the dominance of non-H-2 susceptible alleles of the SJL/J strain (Bureau *et al.* 1992). These observations prompted us to screen the entire genome of a set of (SJL/J × B10.S)$F_1$ × B10.S backcross animals with polymorphic markers and led us to identify one locus that controls viral persistence in the telomeric region of chromosome 10, close to the *Ifng* locus ($P < 0.0002$). However, this locus does not explain the whole difference of susceptibility between the SJL/J and B10.S

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mice, and another susceptibility locus was tentatively mapped to the telomeric region of chromosome 18, close to the *Mbp* locus ($P < 0.002$; Bureau *et al.* 1993). Two observations make *Ifng* and *Mbp* good candidate genes for the control of viral persistence. First, when the gene coding for the interferon gamma receptor is inactivated, resistant 129/Sv mice become highly susceptible to persistent infection and demyelination (Fiette *et al.* 1995). Second, the shiverer mutation, a deletion in the *Mbp* gene, has a profound effect on the phenotype of C3H mice, a strain of intermediate susceptibility. Homozygous mutants are completely resistant whereas heterozygous mutants are highly susceptible (Bihl *et al.* 1997).

Fourteen congenic lines, 9 with different telomeric regions of chromosome 10 and 5 with different telomeric regions of chromosome 18, have now been constructed and their susceptibility to Theiler's virus persistence has been studied. Although the presence of a susceptibility locus in the telomeric region of chromosome 18 was not confirmed, two susceptibility loci, *Tmevp2* and *Tmevp3*, were localized respectively more centromeric and more telomeric than the *Ifng* locus on chromosome 10. The mode of inheritance of these loci was analyzed by intercrossing the congenic lines and crossing them with the SJL/J strain. An effect of sex was observed, with males being more infected than females.

## MATERIALS AND METHODS

**Mice:** SJL/J mice were purchased from Janvier, France. B10.S mice were obtained from the Jackson Laboratory and the INSERM U93 animal facility (Hôpital Saint Louis, Paris, France). All animals were bred in the animal facility of the Pasteur Institute. B10.S-derived genetic intervals were transferred onto a susceptible (SJL/J) background by 10 successive backcrosses (N11) followed by intercrossing to establish homozygous founders. Conversely, an SJL/J-derived genetic interval was transferred onto a resistant (B10.S) background by 8 successive backcrosses (N9) followed by intercrossing to establish homozygous founders. At each generation during backcrossing, all the polymorphic markers described below were analyzed in the progeny to detect recombination and to derive as many congenic lines as possible. For testing phenotypes, 3- to 4-week-old mice were inoculated intracranially with $10^4$ pfu of the DA strain of Theiler's virus in 40 μl of phosphate buffer saline (PBS) and killed 45 days later.

**Microsatellite genotyping:** DNA was extracted from tail biopsies of weanling mice by standard methods. Primers for polymorphic microsatellite markers were obtained from Genset or synthesized by the Pasteur Institute Facility. Sequences of markers *D10Mit68*, *D10Mit70*, *D10Mit135*, *D10Mit122*, *D10Mit73*, *D10Mit233*, *D10Mit180*, *D10Mit74*, *D10Mit234*, *D10Mit14*, *D10Mit164*, *D10Mit237*, *D10Mit151*, *D10Mit271*, *D18Mit8*, *D18Mit3*, *D18Mit127*, *D18Mit4*, and *D18Mit144* are available at http://www.genome.wi.mit.edu/cgi-bin/mouse/index/and that of marker *D18Nds1* (*Mbp*) at http://www.well.ox.ac.uk/~plyons/MouseData/NDSMicrosTop.html/. The primers used to detect a microsatellite intragenic to the *Myf6* gene were 5′-GAAAGGGCACTGGGCTGTAC-3′ and 5′-CGCCGGATTTGGCTGTTGCT-3′. The *D10Pas4* marker detects a deletion in the promoter of the *Ifng* gene present in the SJL/J strain (P. Monteyne, F. Bihl, F. Levillayer, M. Brahic and J. F. Bureau, unpublished results). Genotyping was done on 150 ng of genomic DNA. DNA was amplified by PCR with a Gene Amp kit (GIBCO-BRL, Gaithersburg, MD) in a 9600 reactor (Perkin Elmer-Cetus, Norwalk, CT). After 2 min of denaturation at 94°, the DNA was submitted to 40 cycles of amplification under the following conditions: 94°, 30 sec; 55°, 30 sec; and 72°, 15 sec.

**Quantification of viral RNA in the spinal cord:** The genotype of each infected congenic mouse was confirmed before analysis of its phenotype. Forty-five days after inoculation, anesthetized mice were perfused through the left ventricle with 20 ml of PBS and the spinal cord was immediately removed. Total RNA was extracted by the procedure of Chomczynski and Sacchi (1987) and quantified by spectrophotometry. For each mouse, four serial fivefold dilutions of total spinal cord RNA, starting with 10 μg per sample, were dotted on Hybond C-extra filters (Amersham, Arlington Heights, IL) and hybridized with a $^{32}$P-labeled cDNA probe specific for either the 5′ extremity of TMEV genome or for β-actin mRNA. For each sample, the highest dilution that gave a signal was used as a measure of viral RNA content and was expressed as a score varying from 0 to 4 (Tables 2 and 3) or as a dilution factor varying from 1 to 125 (Figures 2, 3, and 4). Two procedures were used to normalize the data. First, reference samples from previous blots were analyzed repeatedly to adjust for small variations of hybridization efficiency from blot to blot. Second, the persistence score of each sample on a blot was adjusted, if needed, according to the amount of RNA dotted on the filter, defined by the hybridization signal obtained with the β-actin probe.

**Statistical analysis:** The mean and standard error of mean of the amount of viral RNA in the spinal cord were calculated for each group of mice (15–46 animals per group). The analysis of variance or the unlinked Student's *t*-test from the Statview F-4.5 package were used to analyze the results. For multiple tests (Table 2), the value of the *P* threshold was corrected according to the formula $P = 1 - (1 - P_t)^{1/n}$, where $P_t$, the standard threshold for pointwise statistics, is equal to 0.05 (*) or 0.01 (**) and $n$ is the number of tests.

To test the two-loci model, the variability of viral persistence among mice congenic for chromosome 10 was analyzed as a function of strain status after the animals were classified according to their sex. The analysis was performed either under no assumption, using a classical one-way analysis of variance, or after classification of the lines according to the two-loci model. For the latter, mice were divided in groups according to their sex and to the number of resistant B10.S alleles for each locus predicted by the model. For each group, variability of viral persistence was studied depending on strain status. In practice, the sums of squares and the degrees of freedom due to the strain status and to the residual were calculated for each group and added to perform a one-way analysis of variance.

## RESULTS

**Construction of congenic lines:** Two loci controlling the persistence of Theiler's virus have been identified by analyzing the progeny of an (SJL/J × B10.S)$F_1$ × B10.S backcross. One was located in the telomeric region of chromosome 10 and the other one tentatively located in the telomeric region of chromosome 18 (Bureau *et al.* 1993). For the present study, 16 newly identified microsatellites (14 in the telomeric region of chromosome 10 and 2 in the telomeric region of

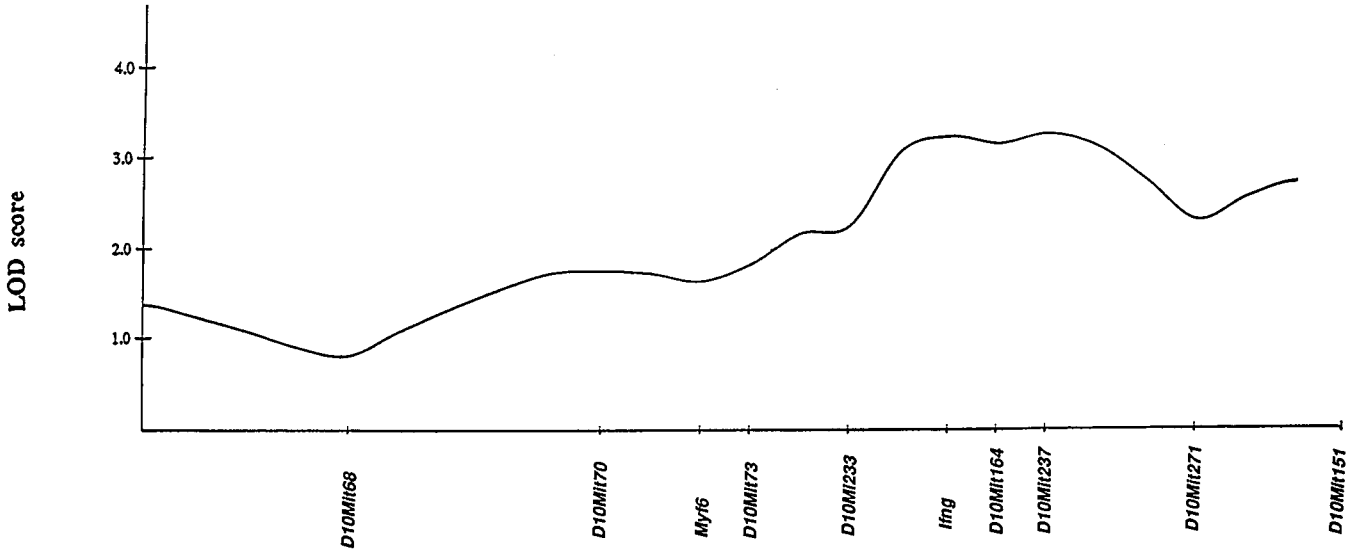


Figure 1.—Localization of QTL on chromosome 10. Data published previously for 79 (B10.S × SJL/J)$F_1$ × B10.S animals (Bureau *et al.* 1993) were reanalyzed after the addition of the results obtained from newly described markers. The analysis was performed with the Mapmaker program (Lander *et al.* 1987). A single marker is indicated when loci cosegregated. Marker *D10Mit135* cosegregates with *D10Mit70*; markers *D10Mit180*, *D10Mit74*, *D10Mit234*, and *D10Mit14* cosegregate with *Ifng*.

chromosome 18) were added to the initial backcross study; the Mapmaker program was used to refine the localization of genetic intervals containing QTL (Lander *et al.* 1987). The chromosome 10 QTL was most probably located in a 10- to 15-cM genetic interval (Figure 1) and the one on chromosome 18 in a 5-cM genetic interval (data not shown).

Congenic lines were obtained from an (SJL/J × B10.S)$F_1$ by successive backcrosses to the susceptible SJL/J parent with selection for B10.S genetic intervals containing the QTL. When a new combination of resistant alleles occurred, the corresponding animal was isolated and crossed further with the SJL/J strain to obtain a new congenic line. After 10 backcrosses, B10.S chromosomal segments were fixed at homozygosity by intercrossing heterozygous animals. Thus, 9 lines of congenic mice were obtained for the telomeric end of chromosome 10 and 4 for the telomeric end of chromosome 18. Another line was constructed, which had an SJL/J segment for the most distal part of chromosome 18

**TABLE 1**

**Genotypes of the congenic lines**

| Line | | Marker | | Minimal size of the congenic region (in cM) | |
|---|---|---|---|---|---|
| No. | Full name | Proximal | Distal | CC | BC1 |
| 1 | SJL.B10-*D18Mit8-D18Mit144* | *D18Mit8* | *D18Mit144* | 10 | 2.5 |
| 2 | SJL.B10-*D18Nds1-D18Mit4* | *D18Nds1* | *D18Mit4* | 2 | 0 |
| 3 | SJL.B10-*D18Nds1-D18Mit144* | *D18Nds1* | *D18Mit144* | 2 | 0.6 |
| 4 | SJL.B10-*D18Mit8-D18Mit127* | *D18Mit8* | *D18Mit127* | 6 | 0.6 |
| 5 | B10.S-*D18Mit8-D18Mit144* | *D18Mit8* | *D18Mit144* | 10 | 2.5 |
| 6 | SJL.B10-*D10Mit70-D10Mit14* | *D10Mit70* | *D10Mit14* | 6 | 9.1 |
| 7 | SJL.B10-*D10Mit233-D10Mit237* | *D10Mit233* | *D10Mit237* | 5.5 | 5.2 |
| 8 | SJL.B10-*D10Mit70-D10Mit122* | *D10Mit70* | *D10Mit122* | 2 | 2.6 |
| 9 | SJL.B10-*D10Mit180-D10Mit74* | *D10Mit180* | *D10Mit74* | 1 | 0 |
| 10 | SJL.B10-*D10Mit233-D10Mit74* | *D10Mit233* | *D10Mit74* | 3 | 2.6 |
| 11 | SJL.B10-*D10Mit164-D10Mit237* | *D10Mit164* | *D10Mit237* | 0 | 1.3 |
| 12 | SJL.B10-*D10Mit164* | *D10Mit164* | *D10Mit164* | 0 | 0 |
| 13 | SJL.B10-*D10Mit233* | *D10Mit233* | *D10Mit233* | 0 | 0 |
| 14 | SJL.B10-*D10Mit237* | *D10Mit237* | *D10Mit237* | 0 | 0 |

The most proximal and distal markers of the donor genetic interval are shown for each line. The distances between markers are based on the 1997 Mouse Chromosome 10 report (CC) or on genotyping of our original backcross progeny (BC1).

**TABLE 2**

**Amount of viral RNA in the spinal cord of congenic and parental strains 45 days postinoculation**

| Line | | No. of animals | Viral persistence (mean ± SEM) | *P* value |
|---|---|---|---|---|
| No. | Full name | | | |
| | SJL/J | 28 | 2.018 ± 0.157 | |
| 1 | SJL.B10-*D10Mit8-D18Mit144* | 18 | 1.472 ± 0.319 | 0.0972 |
| 2 | SJL.B10-*D18Nds1-D18Mit4* | 28 | 1.571 ± 0.204 | 0.0886 |
| 3 | SJL.B10-*D18Nds1-D18Mit144* | 23 | 1.739 ± 0.245 | 0.3277 |
| 4 | SJL.B10-*D18Mit8-D18Mit127* | 25 | 1.540 ± 0.182 | 0.0516 |
| | B10.S | 22 | 0.068 ± 0.050 | |
| 5 | B10.S-*D18Mit8-D18Mit144* | 16 | 0.031 ± 0.031 | 0.5698 |
| | SJL/J | 28 | 2.018 ± 0.157 | |
| 6 | SJL.B10-*D10Mit70-D10Mit14* | 21 | 1.190 ± 0.203 | 0.0020* |
| 7 | SJL.B10-*D10Mit233-D10Mit237* | 17 | 0.853 ± 0.218 | <0.0001** |
| 8 | SJL.B10-*D10Mit70-D10Mit122* | 23 | 1.304 ± 0.188 | 0.0051* |
| 9 | SJL.B10-*D10Mit180-D10Mit74* | 46 | 1.196 ± 0.149 | 0.0006** |
| 10 | SJL.B10-*D10Mit233-D10Mit74* | 42 | 1.881 ± 0.168 | 0.5741 |
| 11 | SJL.B10-*D10Mit164-D10Mit237* | 20 | 2.000 ± 0.140 | 0.9360 |
| 12 | SJL.B10-*D10Mit164* | 25 | 1.840 ± 0.197 | 0.4801 |
| 13 | SJL.B10-*D10Mit233* | 15 | 2.333 ± 0.232 | 0.2563 |
| 14 | SJL.B10-*D10Mit237* | 20 | 2.075 ± 0.212 | 0.8261 |

The amount of viral RNA is expressed as the score given by a quantitative dot blot assay (see materials and methods). The number of mice studied, the mean, and the standard error of mean are shown. The mean values were compared by the unlinked Student's *t*-test. Comparison was with the parental strain contributing the background. As multiple tests were performed, *P* values were considered as significant (*) if they were $\leq 5.68 \times 10^{-3}$ for the SJL.*D10* series of congenic lines and $\leq 1.27 \pm 10^{-2}$ for the SJL.*D18* series. *P* values were considered as highly significant (**) if they were $\leq 1.12 \times 10^{-3}$ for the SJL.*D10* series of congenic lines and $\leq 2.51 \times 10^{-3}$ for the SJL.*D18* series.

in a B10.S background. The SJL segment was fixed at homozygosity after 8 backcrosses (Table 1). The full names of these 14 lines are given in Table 1. For the sake of simplicity they are referred to as lines 1 to 14, as shown in the Table, in the rest of this article. The level of susceptibility of each line was measured as described in materials and methods. Results were expressed as a dilution factor varying from 1 to 125 and as a score varying from 0 to 4 (Tables 2 and 3).

**Congenic lines for the telomeric region of chromosome 18:** The B10.S intervals of 4 congenic lines with a susceptible (SJL/J) background (lines 1–4) are shown in Figure 2B and Table 1. Although all 4 congenic lines had less viral RNA in their spinal cord than the SJL/J parent, this difference was not statistically significant (Figure 2A, Table 2). The SJL/J genetic interval of line 5, which has a resistant (B10.S) background, is shown in Figure 2B and Table 1. This line was not significantly less resistant than the B10.S parent (Figure 2A, Table 2). Therefore, the analysis of congenic lines for the telomeric region of chromosome 18 did not confirm the presence of a susceptibility locus in the region.

**Congenic lines for the telomeric region of chromosome 10:** We first obtained three congenic lines (lines 6–8) carrying a B10.S chromosome 10 segment in an SJL/J background (Figure 3B and Table 1). All 3 lines were significantly less infected than the SJL/J parent (Figure 3A and Table 2). The differences of level of infection between the lines were not statistically significant. The level of persistent infection for these lines amounted to ~20% of that of the SJL/J parent. On the other hand, these lines were significantly more infected than the resistant B10.S parent. Interestingly, lines 7 and 8, which are both more resistant than the SJL/J parent, do not share B10.S genetic intervals derived from chromosome 10. This indicates that the region contains at least two susceptibility loci, which we named *Tmevp2* and *Tmevp3*. *Tmevp2* is located between markers *D10Mit68* and *D10Mit73*, and *Tmevp3* is between markers *D10Mit73* and *D10Mit271*.

**Refining the localization of the *Tmevp3* locus:** The *Ifng* gene, which codes for interferon gamma and is a strong candidate for the control of viral persistence, is located within the genetic interval containing *Tmevp3*. To narrow down this interval and to examine the possibility that *Tmevp3* might be *Ifng*, we derived 6 additional congenic lines (lines 9–14) with small B10.S segments in an SJL/J background (Figure 3B and Table 1). Line 9 was the only one that was significantly less infected than the SJL/J parental strain (Figure 3A and Table 2). In contrast, the level of infection for lines 10–14 was not significantly different from that observed for the SJL/J strain. Because line 9 is more resistant than line 10, *Tmevp3* must be located in a B10.S genetic interval

**TABLE 3**

**Global analysis of variance for sex, *Tmevp2*, and *Tmevp*3 loci**

| | *F* | d.f. | *P* value |
|---|---|---|---|
| Effect | | | |
| Sex | 9.357 | 1 | 0.0023** |
| *Tmevp2* | 3.119 | 2 | 0.0450* |
| *Tmevp3* | 3.985 | 2 | 0.0192* |
| Interaction | | | |
| Sex × *Tmevp2* | 0.595 | 2 | 0.5517 |
| Sex × *Tmevp3* | 0.607 | 2 | 0.5454 |
| *Tmevp2* × *Tmevp3* | 1.673 | 4 | 0.1548 |
| Sex × *Tmevp2* × *Tmevp3* | 0.498 | 4 | 0.7375 |

*P* values were considered significant if they were ≤0.05 (*) and highly significant if they were ≤0.01 (**). Sum of squares and degree of freedom of the residual are 578.701 and 518, respectively.

particular to line 9. Available markers do not identify such an interval and, therefore, locating *Tmevp3* requires making hypotheses. *Tmevp3* cannot be located between *D10Mit233* and *D10Mit74* because line 9 is more resistant than line 10. On the other hand, *Tmevp3* could be located within the genetic interval defined by markers *D10Mit74* and *(D10Mit234,D10Mit14)—D10-Mit234* and *D10Mit14* cosegregate in all cases—an interval that spans <1 cM. In this case, we need to hypothesize that, within the *D10Mit74-(D10Mit234,D10Mit14)* interval, the crossover that defines the telomeric border of the B10.S interval of line 9 is more telomeric than that which defines this border for line 10. It should be noted that the phenotypes of lines 9 and 10 were found to be statistically different in two independent experiments. Figure 3A and Table 2 show the results obtained by combining the data from both experiments. Interestingly, according to this model, *Ifng* is not in the interval containing *Tmevp3*.

**Allelic segregation of the *Tmevp2* and *Tmevp3* loci:** The data presented so far allow us to propose a model in which two loci controlling viral persistence, *Tmevp2* and *Tmevp3*, are localized at the telomeric extremity of chromosome 10. To test this model, lines 6–10 were crossed with each other and with the SJL/J strain, and a three-way analysis of variance was used to study the effect on viral persistence of allelic segregation for these two loci. The amount of viral genome was analyzed as a function of the allelic form of *Tmevp2* and *Tmevp3* and the sex of the animal (this analysis tests the effect of each factor independently from each other and, additionally, tests for possible interactions between them). The analysis was performed under the assumption that the *Tmevp2* locus is located within the *D10Mit68-D10Mit73* interval and the *Tmevp3* locus is within the *D10Mit74-(D10Mit234,D10Mit14)* interval. It confirmed that viral persistence depends on both the *Tmevp2* and *Tmevp3* loci (Table 3). Furthermore, the

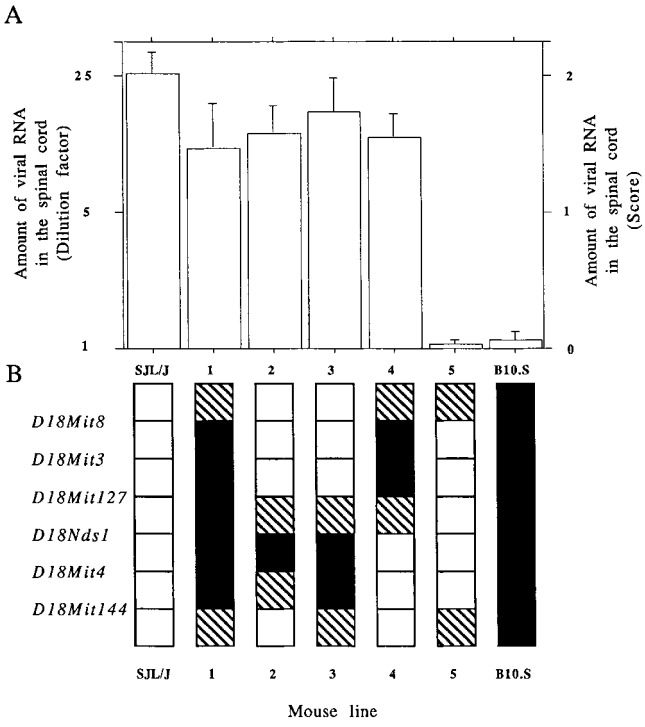


Figure 2.—Susceptibility to viral persistence of five mouse lines congenic for different regions of chromosome 18 and of the parental strains. (A) Amount of viral RNA in the spinal cord, 45 days postinoculation, expressed as the highest RNA dilution that gave a hybridization signal (left ordinate) or the score of viral persistence (right ordinate; see materials and methods). (B) Genotypes. Open squares, regions from the SJL/J parent; solid squares, regions from the B10.S parent; hatched squares, a genetic segment where a crossover occurred. Lanes 1–5 correspond to lines SJL.B10-*D18Mit8-D18-Mit144*, SJL.B10-*D18Nds1-D18Mit4*, SJL.B10-*D18Nds1-D18Mit144*, SJL.B10-*D18Mit8-D18Mit127*, and B10.S-*D18Mit8-D18Mit144*, respectively.

susceptible allele of the *Tmevp2* locus is dominant, or additive, over the resistant allele (Figure 4A), and the susceptible allele of the *Tmevp3* locus is recessive, or additive, over the resistant allele (Figure 4B). Moreover, an effect of the sex of the animals was observed (Table 3), with males being more infected than females. This effect was not observed for mice homozygous for both the *Tmevp2* and *Tmevp3* loci (Figure 4C). No significant interaction was detected among these three factors (Table 3).

## DISCUSSION

The control of the susceptibility to Theiler's virus persistent infection of the CNS is multigenic. Our approach to dissect this complex system has been to select a chromosomal region containing a QTL and to isolate it by successively backcrossing the resistant allele of the QTL to a susceptible background. The congenic lines obtained with this procedure should confirm the existence of susceptibility loci and provide valuable tools to

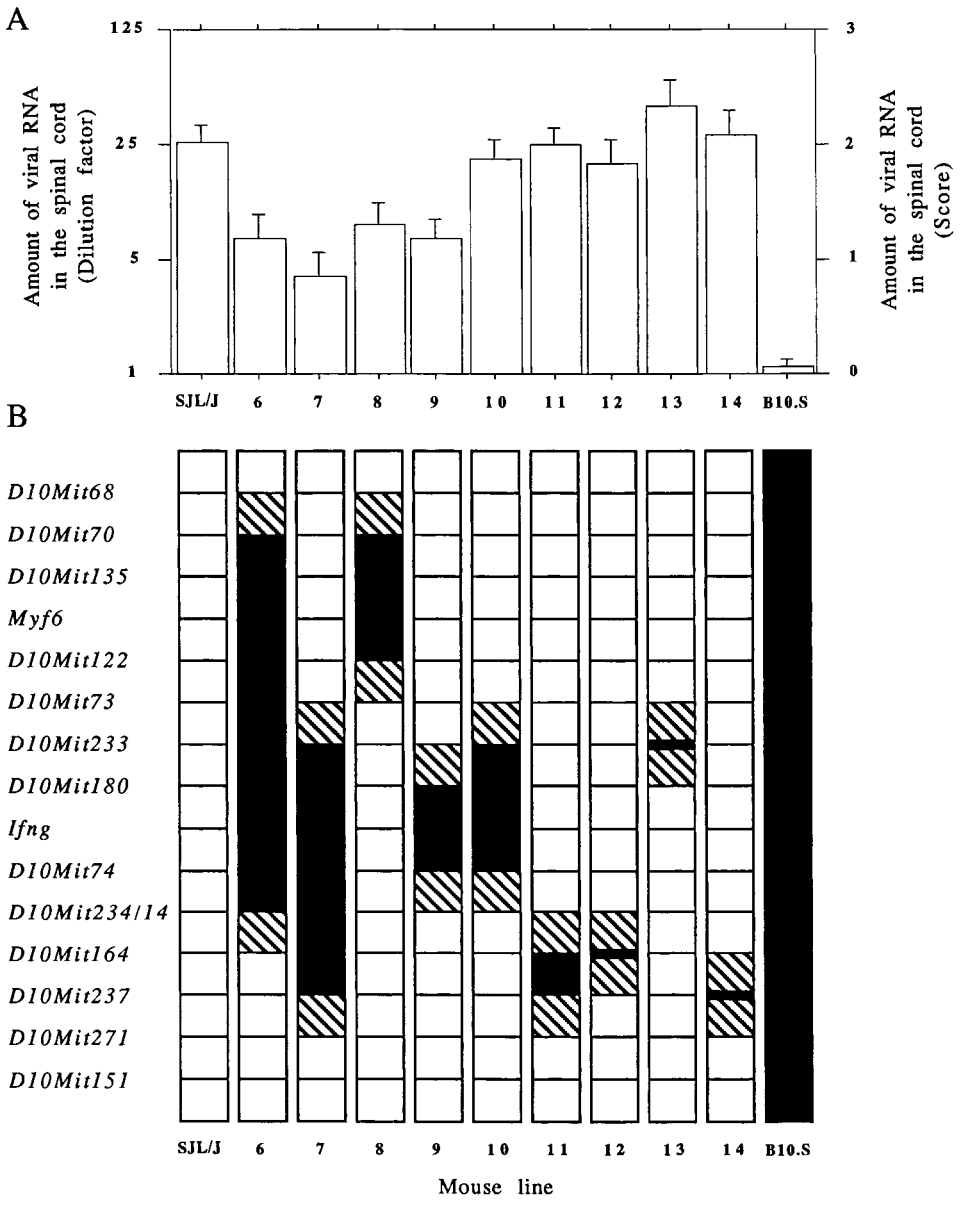


Figure 3.—Susceptibility to viral persistence of nine lines congenic for different regions of chromosome 10 and of the parental strains. (A) Amount of viral RNA in the spinal cord, 45 days postinoculation, expressed as the highest RNA dilution that gave a hybridization signal (left ordinate) or the score of viral persistence (right ordinate; see materials and methods. (B) Genotypes. Open squares, regions from the SJL/J parent; solid squares, regions from the B10.S parent; hatched squares, a genetic segment where a crossing over occurred. Lanes 6–14 correspond to lines SJL.B10-*D10Mit70-D10Mit14*, SJL.B10-*D10Mit233-D10Mit237*, SJL.B10-*D10Mit70-D10Mit122*, SJL.B10-*D10Mit180-D10Mit74*, SJL.B10-*D10Mit233-D10Mit74*, SJL.B10-*D10Mit164-D10Mit237*, SJL.B10-*D10Mit164*, SJL.B10-*D10Mit233*, and SJL.B10-*D10Mit237*, respectively.

identify the mechanism of action of the responsible genes. Eventually, they should make the cloning of these genes possible.

In a previous study, we described two QTL involved in the control of Theiler's virus persistence, one on chromosome 10 and another one on chromosome 18 (Bureau *et al.* 1993). In both cases the QTL cosegregated with the most telomeric marker available at that time on the chromosome (Bureau *et al.* 1993). Several new markers have been described in these two regions since the original study. They were used to refine the QTL localization and the size of the B10.S donor genetic interval to be transferred during backcrossing. More markers were described while backcrossing was under way. Two crossovers that excluded the telomere of chromosome 10 from the B10.S interval of our congenic lines had occurred before we were able to detect them with markers *D10Mit237* and *D10Mit151*. These two events are at the origin of congenic lines 7 and 6, respectively.

The phenotypes of mice congenic for the telomeric part of chromosome 18 did not confirm the existence of a locus that controls persistence in the region. This discrepancy with the localization of a QTL in the region can be explained in several ways. First, the linkage obtained in the genome scan ($P = 0.002$) might have been fortuitous. Second, the B10.S genetic intervals in the congenic lines might be too small to include a susceptibility locus. In particular, if this locus is more distal than our most telomeric marker, *D18Mit144*, it could have been excluded from the selected interval. Third, the effect of the locus might not be seen in the congenic lines because of interactions with other uncharacterized loci. Although a simple form of interaction is excluded

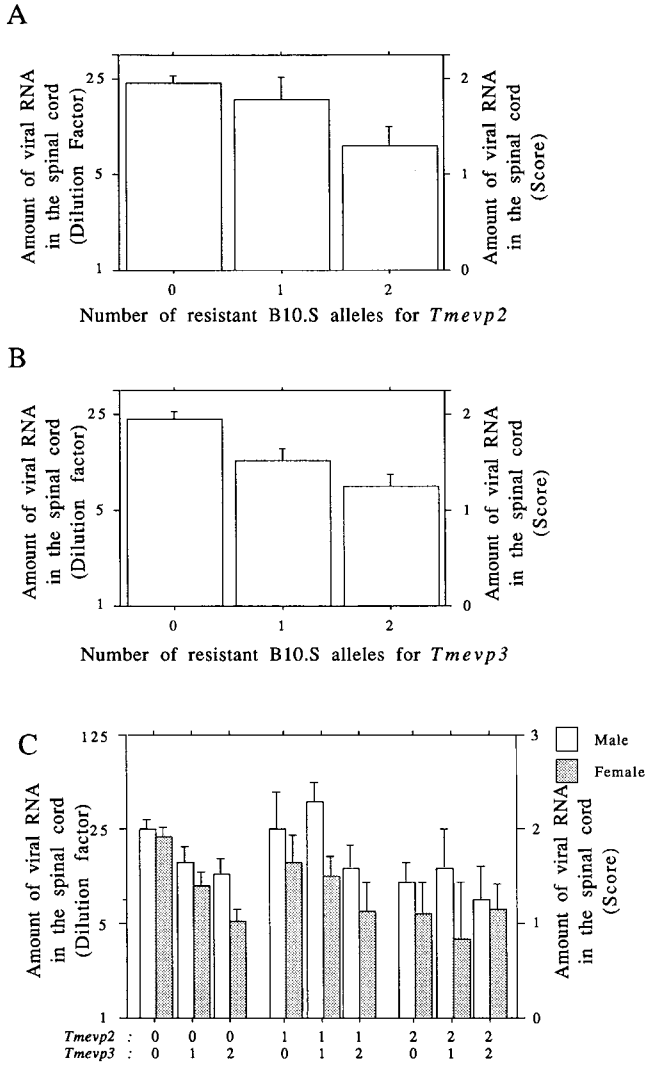


Figure 4.—Allelic segregation of the *Tmevp2* and *Tmevp3* loci and effect of sex. (A) Viral persistence as a function of the number of resistant (B10.S) alleles of the *Tmevp2* locus. Only mice with no resistant (B10.S) allele for the *Tmevp3* locus were used in this analysis. (B) Viral persistence as a function of the number of resistant (B10.S) alleles of the *Tmevp3* locus. Only mice with no resistant (B10.S) allele for the *Tmevp2* locus were used in this analysis. (C) Effect of sex depending on the number of resistant (B10.S) alleles for the *Tmevp2* and *Tmevp3* loci. The amount of viral RNA in the spinal cord, 45 days postinoculation, is expressed as the highest RNA dilution that gave a hybridization signal (left ordinate) or the score of viral persistence (right ordinate; see materials and methods).

because reciprocal lines 1 and 5 have the same level of susceptibility as the SJL/J and B10.S parental strains, respectively (Figure 3, Table 2), the possibility of more complex interactions between loci cannot be ignored.

The study of nine congenic lines for the telomeric region of chromosome 10 allowed us to prove the existence of at least two susceptibility loci in the region. Because of the resistance of line 8, we located the first locus, which we named *Tmevp2*, in a region of ~10.5 cM between markers *D10Mit68* and *D10Mit73*. Determining the position of the other loci required making models. The simplest one, which has been described in results, calls for the existence of only one other susceptibility locus in the region, *Tmevp3*, located between markers *D10Mit74* and *(D10Mit234,D10Mit14)*, within an interval of <1 cM. The model implies that the telomeric extremity of the B10.S interval of line 9 is more telomeric than that of the susceptible line 10. Presently, *D10Mit74* is the most telomeric marker for the B10.S interval of both lines. Therefore, to confirm this model and to characterize *Tmevp3* it will be essential to identify polymorphic markers in the *D10Mit74-(D10Mit234, D10Mit14)* interval and to look for different crossover in this interval in lines 9 and 10. Other models can be envisioned. However, in their simplest form, they require the existence of three additional susceptibility loci besides *Tmevp2*, and interactions between them. Because of their complexity and because the statistical analysis of our data, described below, is congruent with the simple model, we do not favor these models.

To test the simple two-loci model, the "resistant" congenic lines described in Figure 3 were crossed with each other and with the SJL/J parent, and the phenotypes of these mice were measured. When the data for all chromosome 10 congenic lines and for these crosses were analyzed without taking any model into consideration, the effect of the strain status on the standard error was highly significant ($F(49,502) = 2.047$; $P < 0.0001$). When the data were reanalyzed with these lines and their crosses classified according to the simple two-loci model, the effect of the strain status on the standard deviation was drastically reduced ($F(31,443) = 1.491$; $P = 0.0460$). Therefore, most of the variability of viral persistence among congenic lines is explained by the model (57% of the total standard deviation is explained by *Tmevp2* and *Tmevp3*).

An analysis of variance of our data according to the two-loci model showed an effect of sex, of *Tmevp2* and *Tmevp3*, and no interaction between these factors (Table 3). Males are more infected than females. A greater susceptibility of males to clinical disease has been reported previously in the analysis of a different cross (Kappel *et al.* 1990). This effect of sex was not observed in our original backcross analysis, even for markers in the telomeric region of chromosome 10. In the present study, the effect is seen when a crossover has occurred between the *Tmevp2* and *Tmevp3* loci (Figure 4C). Because both loci are <15 cM apart this may have occurred too rarely in the original backcross to allow detecting the effect of sex. Although no effect of sex was detected in analyzing the (SJL/J × B10.S)$F_1$, this effect was obvious in the present work for mice heterozygous at both the *Tmevp2* and *Tmevp3* loci (Figure 4C). As shown in Figure 1, the analysis of the (SJL/J × B10.S)$F_1$ × B10.S backcross located *Tmevp3* rather accurately, but did not detect *Tmevp2*. The study of the mode of inheritance of these loci in crosses between congenic lines and be-

tween a congenic line and the SJL/J strain showed that both loci should have been detected in the backcross [In Figure 4C, compare mice with the (1,1) combination of resistant alleles with mice with the (1,2), (2,1), and (2,2) combinations]. The fact that *Tmevp2* was not detected suggests that it interacts with other unidentified loci.

Interestingly, this study showed that *Ifng*, a strong candidate gene for controlling viral persistence, is excluded from the regions containing *Tmevp2* and *Tmevp3*. This is consistent with recent data from our laboratory on the expression pattern of the *Ifng* gene during the early phase of the infection (P. Monteyne, F. Bihl, F. Levillayer, M. Brahic and J. F. Bureau, unpublished results). Finally, it is interesting to note that our system illustrates once again how, in multigenic diseases, several susceptibility loci are often located within genetic intervals where a single QTL had originally been detected (Podolin *et al.* 1997; Melanitou *et al.* 1998).

In summary, the use of congenic lines allowed us to exclude *Ifng*, a strong candidate gene, from the control of the persistent infection by Theiler's virus. Instead, our data led us to propose the existence of at least two susceptibility loci in the region. This study gives an example of the complexity of the genetic control of infections and illustrates the power of congenic mice to dissect such a control.

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